AMENDMENTS TO THE SPECIFICATION:

Please replace the paragraph on page 5, beginning at line 3 as follows:

A method of inactivating enveloped viruses in a preparation containing recombinant adenoviruses as active ingredients has now been developed which uses the solvent tri-n-butyl phosphate (TNBP). Moreover, the effect of different variables has been studied in order to define the experimental conditions most appropriate for preserving the infectivity of the recombinant adenoviruses and so as to become integrated in an overall method of purification. The examples which follow show that the action of 0.1 to 0.6% TNBP and of 1% to 2% ~~Tween 80~~ Tween® 80 for 4h at room temperature makes it possible to significantly reduce the quantity of enveloped viruses (reduction by a factor of at least 4 log units) while preserving the integrity of the adenoviral particles (yield of at least 80%, or even greater than 100%). The beneficial effect of the method according to the invention for reducing the aggregates which spontaneously form between the virions and hamper the infectivity of the viruses has also been demonstrated.

Please replace the paragraph on page 9, beginning at line 28 as follows:

Although the choice of the solubilizing agent is not limited, there may be mentioned in particular the polyoxyethylene derivatives of fatty acids or of their esters. The preferred solubilizing agents include Tween (in particular ~~Tween 20 or 80~~ Tween® 20 or 80), Triton (in particular X-100), PEG (in particular PEG 400), sodium cholate, sodium deoxycholate, octyl β-D-glucopyranoside and N-dodecyl-N, N-dimethyl-2-ammonio-1-ethane sulphonate. ~~Tween 80~~ Tween® 80 is most preferred. The combination TNBP and ~~Tween 80~~ Tween® 80 is preferred in the context of the invention.

Please replace the paragraph on page 10, beginning at line 1 as follows:

In the case where this embodiment is selected, the final concentration of solubilizing agent to be used may vary in a wide range. As a guide, is may be

between 0.001% and 10%, in particular between 0.01% and 5% and preferably between 0.1% and 2%. As regards ~~Tween 80~~ Tween® 80, the optimum concentration is between 0.5% and 2%. The combinations TNBP 0.6% and ~~Tween 80~~ Tween® 80 2% as well as TNBP 0.3% and ~~Tween 80~~ Tween® 80 1% are particularly preferred.

Please replace the paragraph on page 27, beginning at line 18 as follows:

The quality, that is to say the degree of purity of the viral preparation, may be monitored throughout the method of preparation according to the invention by determining the residual concentration of the contaminants and the functionality of the non-enveloped virus of interest. In the first case, and this being the preferred embodiment, the disappearance of ~~Tween 80~~ Tween® 80 (or polysorbate 80) after step (f) may be assessed by the method recommended in the European Pharmacopoeia (1997, p. 1372-1373) with the aid of potassium thiocyanate and chloroform. The quantity of TNBP present in the viral preparation may be titrated by the gas chromatography technique as disclosed in the Horowitz et al. (1985, Transfusion 25, 516-522). The residual concentration of the proteins may be measured by any technique for assaying proteins. A suitable technique is that of BCA (bicinchoninic assay) (kit Micro BCA Protein Assay Reagent Kit; Pierce ref 23235). As regards the viral active ingredient, the number of complete particles is determined by the spectrometry at a wavelength of 260 nm in the presence of SDS (see Shabram et al., 1997, Human Gene Therapy 8, 453-465). The functionality of the non-enveloped virus is generally determined by its infectious capacity, for example by titrating the number of infectious units (see Lusky et al., 1998, J. Virol. 72, 2022-2032). In the case of a recombinant virus, it is also possible to evaluate the expression of the recombinant gene, after infecting the target cell, by fluorescence, immunological methods (ELISA, RIA and the like), immunoenzymatic methods (Western and the like), staining techniques or luminescence, and the like.

Please replace the paragraph on page 35, beginning at line 27 as follows:

At this stage, the viral preparation is very viscous because of the release of the genomic DNA following the cell disruption. There is added to the viral preparation one volume of a buffer allowing optimum action of benzonase and consisting of 100 mM Tris, 4 mM $MgCl_2$, 4% sucrose, pH 8.5, to which the solubilizing agent ~~Tween 80~~ Tween® 80 (Merck reference 8-22187-1000) has been added at a concentration of 2%. The mixture is stirred at room temperature before adding the benzonase in an amount of 50 U/ml (Merck reference 101697) and the reaction is allowed to continue for 1 to 2 h at room temperature and with stirring.

Please replace the paragraph on page 36, beginning at line 9 as follows:

The step of inactivating the enveloped viruses is carried out by the action of TNBP at a final concentration of 0.3%. To do this, the filtrate is diluted volume for volume in a 50 mM Tris buffer solution containing 2 mM $MgCl_2$, 2% sucrose, _350 mM NaCl and 0.6% TNBP (Aldrich 24-049-4), pH 8.5. It is also possible to add to the filtered viral preparation 9 volumes of a more concentrated buffer (50 mM Tris, 2 mM $MgCl_2$, 2% sucrose, 1.82 M NaCl and 3% TNBP, pH 8.5). It should be noted that the saline conditions used (250 mM NaCl final) correspond to the equilibration conditions for ion-exchange chromatography. The action of ~~TNBP/Tween 80~~ TNBP/Tween® 80 is allowed to continue, with stirring (500 rpm), at room temperature for 3 h or at 4°C for 4 h.

Please replace the paragraph on page 38, beginning at line 18 as follows:

EXAMPLE 3: Inactivation of enveloped viruses

3.1 Validation on a retrovirus preparation

The efficiency of the method of inactivation proposed in the present invention is evaluated on recombinant retroviruses expressing the LacZ marker gene encoding the enzyme β-galactosidase. A 20 F500 culture of 293 cells, is prepared. After centrifugation for 8 min at 3000 rpm, the cells are taken up in serum-free medium.

The preparation contains 3 x 10^7 cells/ml in a volume of 25 ml. The cells are disrupted in a Silverson and then centrifuged for 10 min at 3500 rpm in order to remove the debris. The preparation is then separated into 2, a first half being diluted volume for volume in the benzonase buffer (100 mM Tris, 4 mM $MgCl_2$, 4% sucrose, pH 8.5) in the absence of β-cyclodextrin whereas the second half is treated in a similar manner but in the presence of 3% β-cyclodextrin (1.5% final). The samples are clarified by cascade filtration on Minisart filters (Sartorius) of 5 µm (reference 17594Q), of 1.2 µm (reference 17593Q) and 0.8 µm (reference 17592Q). Each sample is then treated with one volume of 50 mM Tris, 2 mM $MgCl_2$, 2% sucrose, 450 mM NaCl, 0.6% TNBP and 2% ~~Tween 80~~ Tween® 80, pH 8.5. The retroviral particles are introduced at a final concentration of 1.5 x 10^6 infectious particles/ml. The retroviral particle titre is determined after 15 sec, 20 min, 1 h, 2 h and 4 h of incubation either at 4°C or at room temperature. The titration is carried out by counting the blue cells according to the standard methodology (see for example US 5,747,323).

Please replace the paragraph on page 40, beginning at line 1 as follows:

A small scale (18 ml) adenoviral preparation is prepared according to the protocol used in example 1. After clarification by depth filtration in 4 successive steps, 2 ml of a particles solution of BVD are introduced. Then, the inactivation step is carried out in presence of a final concentration of 0.3% TNBT and 1% ~~Tween 80~~ Tween® 80. The titre in BVD and infectious adenoviral particles is determined after 0 min, 15 min, 60 min and 120 min of incubation at room temperature.

Please replace the paragraph on page 41, beginning at line 12 as follows:

At the clarified viral preparation is added a volume of a buffer allowing the optimum action of benzonase and consisting of 100 mM Tris-HCL, 4 mM $MgCl_2$, 4% saccharose, pH 8.5, further comprising ~~Tween 80~~ Tween® 80 (Merck reference 8-22187-1000) at a concentration of 2%. The mixture is stirred at room temperature before adding the benzonase in an amount of 10 U/ml (Merck reference 101697) and the reaction is allowed to continue for 2 h at room temperature and with stirring (500

rpm). The clarified viral preparation may also be subjected to the simultaneous action of the benzonase (degradation step of the DNA) and of ~~TNBP/Tween 80~~ TNBP/Tween® 80 (inactivation of the enveloped viruses). To do so, TNBP (Aldrich 24-049-40) is added to the precedent preparation at a final concentration of 0.3%. The action of ~~TNBP/Tween 80~~ TNBP/Tween® 80 continues with stirring (500 rpm). The titre in infectious units determined after each essential step of the process is summarized in the following table.

Please replace the paragraph on page 42, beginning at line 4 as follows:

EXAMPLE 5: Validation of an adenoviral preparation contaminated by VSV virus

A small-scale adenoviral preparation is prepared according to the protocol used in example 4. After breaking and clarification by depth filtration, a solution of VSV particles (ATCC VR-158; 9.9 $\log_{10}$ TCTID50) is introduced in the adenoviral preparation (2.5×10^{10} ui). Then, the inactivation step is carried out in presence of a final concentration of 0.3% TNBP and 1%
~~Tween 80~~ Tween® 80 simultaneously as the nucleic acids degradation step in presence of 10 U/ml of benzonase (Merck reference 101697). The infectious VSV particles titre is determined on VERO cells according to known techniques (Virology Methods Manual 1996, pp. 35-40, Ed. Mahy and Kangro, Academic Press Ltd. London) after 0 min, 30 min, 60 min and 120 min of incubation at room temperature.